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# Lab-on-DVD: standard DVD drives as a novel laser scanning microscope for image based point of care diagnostics

Harisha Ramachandraiah,[a] Mary Amasia,[a] Jackie Cole,[b] Paul Sheard,[b] Simon Pickhaver,[b] Chris Walker,[b] Valtteri Wirta,[c] Preben Lexow,[c] Richard Lione[b] and Aman Russom*[a]

Received 11th December 2012, Accepted 1st February 2013

DOI: 10.1039/c3lc41360h

**www.rsc.org/loc**

We present a novel ''Lab-on-DVD'' system and demonstrate its capability for rapid and low-cost HIV diagnostics by counting CD4+ cells isolated from whole blood. We show that a commercial DVD drive can, with certain modifications, be turned into an improved DVD-based laser scanning microscope (DVD-LSM). The system consists of a multi-layered disposable polymer disc and a modified commercial DVD reader with rotational control for sample handling, temperature control for optimized bioassay, a photodiode array for detection, and software for signal processing and user interface – all the necessary components required for a truly integrated lab-on-a-chip system, with the capability to deliver high-resolution images down to 1 μm in size. Using discs modified with antibodies, we specifically captured CD4+ cells from whole blood, demonstrating single cell resolution imaging. The novel integrated DVD platform with sub-micron image resolution brings, for the first time, affordable cellular diagnostic testing to the point-of-care and should be readily applicable at resource-limited settings.

## Introduction

Worldwide health-care clinics, especially in developing countries, urgently require improvised methods for the detection of disease etiologies in resource-limited settings. Such rapid and easy-to-use diagnostics are especially necessary during the field trials of infectious/communicable diseases such as malaria, tuberculosis and HIV. The pandemic due to HIV/AIDS continues to be one of the leading causes of mortality, affecting more than 34 million people globally.[1] The HIV virus primarily infects the CD4+ T lymphocytes and so, the counting of CD4+ T lymphocytes has become the single most important test used in HIV diagnosis and treatment. In healthy adults, the absolute number of CD4+ T cells $\mu L^{-1}$ of blood is approximately 800–1000 cells $\mu L^{-1}$; whereas the number of CD4 cells inexorably declines during HIV infection due to progressive damage to the immune system. This difference generally determines the eligibility to initiate treatment: a CD4 cell count below 200 cells $\mu L^{-1}$ elicits the diagnosis of AIDS, along with the prescription of antiretroviral treatment (ART) and prophylaxis against opportunistic infections. Higher CD4 cell count thresholds of 350 or 500 cells $\mu L^{-1}$ are widely used to increase the intensity of monitoring. After initiation of treatment, it is advised to monitor the CD4 cell counts (four times per year) for life, in which this monitoring is a critical event for all HIV-infected patients.

Although drug prices have decreased by more than 90%, the lack of an appropriate CD4 count is a critical bottleneck preventing the expansion of efforts to address the AIDS pandemic. The current reference method, flow cytometry is expensive in terms of equipment and reagents and the technical requirements for operation have reached their limit at resource-scarce settings. In addition, flow cytometry is limited to laboratory use, which means samples from rural areas have to be transported to sophisticated urban laboratories making dissemination and follow-up care difficult.[2] Hence, point of care (POC) testing could improve the disease management in rural areas and/or technically under developed geographical locations.

In bringing affordable cellular diagnostics to the POC, microfluidics and the concept of lab-on-a-chip continue to contribute toward this successfully emerging field that aims to integrate complex biochemical analyses into automated POC systems. One of the most promising applications for these microfluidic systems is in POC blood analysis, which require significant sample handling, storage, and preparation steps. Several distinct components are part of any device for blood analysis: sample collection and introduction, sample preparation, analyte separation, analyte detection, and signal processing.[3,4] Practical applications in POC diagnostics, particularly

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[a] *Science for Life Laboratory, Div. of Proteomics and Nanobiotechnology, KTH Royal Institute of Technology, Stockholm, Sweden. E-mail: aman@kth.se*

[b] *Plarion LTD, Cambridge, United Kingdom*

[c] *Lingvitae AS, Oslo, Norway*

in global health applications, require that each of these steps be miniaturized and integrated. While there has been significant progress in applying miniaturized technologies to isolate CD4+ T lymphocytes based on immunoassays using antibodies as part of specific cell capturing and labeling,[5–8] manual sample handling, external detection and sample processing have limited the implementation of these methods in resource-scarce settings. Towards this, we propose here a low-cost DVD platform capable of integrating sample handling and detection for POC blood diagnostics.

The centrifugal (CD) microfluidic platform, with its intrinsic pumping and valving mechanisms for non-contact liquid handling is an ideal choice for such an integrated POC system.[9–11] The CD platform has been utilized in many biomedical applications including sample preparation,[12–14] immunoassay,[15–17] biochemical analysis,[14] and PCR amplifications[18,19] While centrifugal microfluidic systems are adept at integrating sample preparation steps with bio-analyses, integrated detection is a complex task requiring a rotating platform. The rotating disc must be very flat and the detecting mechanism must be capable of self-alignment with a particular spot on the disc surface. Nevertheless, some platforms have reduced this complexity by performing detection while stationary, or have reverse engineered a disposable disc to match with the detection components of an existing commercial system.[19,20]

Alternative detection platforms based on existing infrastructure in optical disc readers for the detection of biological samples have recently gained attention.[16,21–24] The first demonstration utilizing a commercial compact disc drive as a detection system was reported by Kido *et al.*,[16] where biological molecules were immobilized onto the polycarbonate disc surface and the drive's error detection process was used to determine the extent of binding of streptavidin to the surface. Recently, a similar strategy was reported for counting particles and cells.[24] Using an alternative approach, Lange *et al.* converted the pickup unit inside a compact disc drive to a laser scanning microscope to monitor the light reflection from gold nanoparticle-stained immunoassays microcontact-printed on compact disc surfaces.[25] Maquiera *et al.* integrated external photodetectors above the surface of a commercial DVD reader for immunoassay applications.[23] While these existing detection systems show great potential at low cost, there is often a compromise between resolution and disc functionality. In this paper, we bring together the best of both worlds: the ability to manufacture closed lab-on-a-CD systems with an integrated detection of a sub-micron imaging resolution.

Here, we report an integrated DVD platform consisting of a multi-layered disposable polymer disc and modified commercial DVD drive with integrated fluid propulsion and detection capabilities. We show that a commercial DVD drive can be turned into an improved DVD-based laser scanning microscope (DVD-LSM) for the detection of biomolecules on the surface of the disc. Specifically, the detection is based on the increased light scattering of captured biomolecules onto the DVD surface. The optical half discs (DVDs) are bonded to a top fluidic polymer disc enabling sample processing. Using microparticles, we demonstrate imaging using the DVD platform, with a resolution down to 1 μm. Using discs functionalized with anti-CD4 antibodies, we further explored the capability to establish a cell-based HIV diagnostic assay by specific capture of CD4+ cells isolated from whole blood. To the best of our knowledge, this is the first integrated CD system that combines robust fluidic sample handling with an integrated bioassay detection with sub-micron image resolution.

## Materials and methods

### CD design and fabrication

The microfluidic CD device consists of two half-disc substrates bonded together using an UV-cured adhesive. The DVD-ROM substrate is a *ca.* 0.6 mm thick disc that contains all the operational information required to read the disc using a standard DVD drive, including a spiral groove of 0.74 μm track pitch. These half-discs were manufactured with injection-molded polycarbonate substrates (Eximpo/Northern Star, CZ) and function to focus the incident light at the reflector surface. To provide chemical stability and reproducible surface fuctionalization, the surface of the DVD-ROM is coated with a 10 nm thick layer of $SiO_2$. The second half-disc-substrate contains the fluidic microchannels, access through-holes and any other fluidic reservoir designs. This substrate can be fabricated through injection molding, or as pressure sensitive adhesive (PSA) assemblies; in this paper, injection molded channel disc substrates were used. The injection molded polycarbonate channel disc was aligned and screen printed with an UV-curable adhesive layer. The channel features are protected *via* a positive mask, which is positioned above the disc surface, and allows adhesive to be deposited in only the desired non-channel regions.

The two half-substrates were then aligned, placed into a vacuum lamination instrument and pressed together to ensure a uniform, bubble-free layer of UV adhesive. Finally, the assembly was exposed to UV light for 20 s to completely cure the adhesive and form a permanent bond between the two plastic layers.

### DVD system design

In this paper, we show that a commercial DVD drive can be turned into a DVD-based laser scanning microscope (DVD-LSM) for cell-based assays. The DVD-LSM operates in a similar manner to a standard DVD drive, with an additional photodetector module positioned above the disc surface. In this way, cells or other absorbance-based reactions can be detected *via* the decreased absorbance or scattered light reaching the two photodetectors from DVD laser light source.

The integrated DVD system consists of a standard DVD drive, secondary motor, thermocouples and an array of photodiodes with an integrated printed circuit board (PCB) to allow automation and control *via* a computer (Fig. 1A). The internal DVD drive motor spins the disc at 2 ×, 4 ×, 6 ×, and 8 × constant linear velocity (CLV) (∼1200, 2400, 3600 and

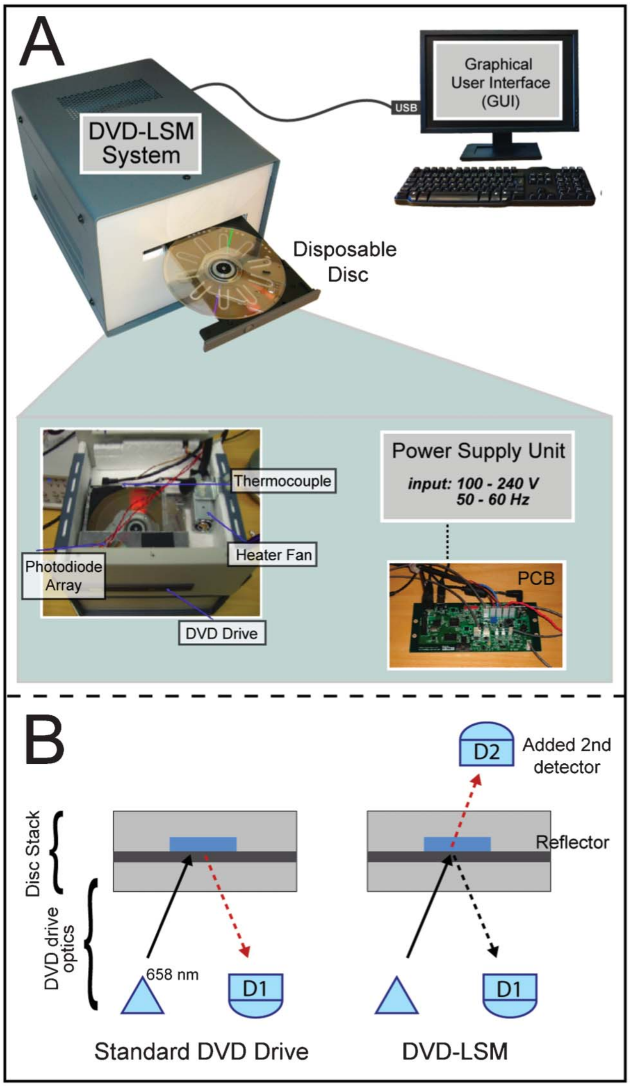

**Fig. 1** Optical disc based laser scanning microscope (DVD-LSM). (A) Overview of the DVD-LSM platform. The hardware includes an external motor for rotational control, a thermocouple for temperature control and an array of photodiodes for photodetection, integrated into the prototype and controlled *via* the user interface on a PC. The unit is driven by software on a PC, and data is transferred *via* USB. (B) The principle of DVD-LSM: A second photo detector is added to a standard DVD reader to record the amount of scattered light or absorbance, with collected data assembled into image files.

4800 RPM), while the external motor can be programmed to spin at arbitrary RPM. The DVD-LSM system is equipped with a Cirrus fan heater to control the temperature up to approximately 80 °C. A proportional-integral-derivative (PID) temperature controller and three embedded thermocouples are used to monitor and control the enclosure temperature.

A PCB has been designed and manufactured to provide integrated control of the supplementary functionalities of the integrated system that include analog to digital conversion, PID temperature control, connectivity to the photodetector (PD) board, and connectivity to the PC, which was powered by a standard electrical plug and controlled through a USB interface with a computer.

The principle of the readout system is illustrated in Fig. 1B. The key difference compared to a standard DVD drive is the use of a second photodiode (D2) above the DVD drive that picks up transmitted light through the semi-transparent DVD substrate. This approach enables monitoring of reactions in the microfluidic channel structure placed above the reflector surface, and of objects within the laser focal plane. The fundamental principle of data generation using the DVD-LSM system is based on end-user input *via* a graphical user interface (GUI). The user specifies where to pick up the signal from a known starting position following the spiral track of the disc, and the photodetector (D2) captures the amount of light transmitted through the disc. Software-based processing generates a 2-dimensional image, which can be saved to the computer hard drive for further analysis.

### Surface modification

Before cell binding experiments can be performed, the interior channel surfaces of the disposable disc must be chemically modified to allow surface attachment of cells or particles. Prior to bonding, the surface of the lower disc was chemically deposited with silane *via* solution immersion method. Briefly, the discs were immersed in freshly prepared 0.5% GOPTS solution, baked at 120 °C for 20 min and stored under nitrogen.

After bonding to the channel disc, channels were functionalized by filling neutravidin solution (pH 7), incubated for an hour at room temperature and washed three times with 1% BSA. Finally, the channels were filled with a solution containing biotinylated anti-CD4 antibodies (Thermo Scientific, Germany) and incubated for two hours at room temperature. The channels were then connected to tubing *via* Nanoports (IDEX Health and Science, Germany) and washed by pumping 1% BSA solution at 10 μl $\text{min}^{-1}$ for 10 min using a syringe pump (PHD 2000 - Harvard Apparatus).

### Cell line and blood experiments

The T2 cell line samples were cultured in Iscove's Modified Dulbecco's Medium containing 10% fetal bovine serum (Sigma Aldrich, Germany) and 0.1% non-essential amino acids. Cells were cultured in a $CO_2$ incubator and the medium was exchanged every two to three days through standard cell culture practice. Blood samples from healthy donors were collected (Blood Center, Stockholm, Sweden) in 5 ml EDTA Vacutainer tubes and processed on the day of collection.

For the cell capture experiments, T2 cells were washed, resuspended in PBS and 100 μl of the suspension was pumped into the channel at 3 μl $\text{min}^{-1}$ using a syringe pump (Harvard apparatus, USA). The channels were washed with 1% BSA (w/v) at 15 μl $\text{min}^{-1}$ for 10 min to remove the unbound cells. In the blood experiments, 10 μl of whole blood was pumped through at a flow rate of 3 μl $\text{min}^{-1}$, and then washed with 1% BSA at a flow rate of 15 μl $\text{min}^{-1}$ for 10 min. After washing, cells were stained with Hematoxylin and eosin stain. Hematoxylin stain was pumped at a flow rate of 15 μl $\text{min}^{-1}$ for 10 min and washed with 1× PBS at the same flow rate. Eosin stain was then pumped at 15 μl $\text{min}^{-1}$ for 5 min and washed with 1× PBS to complete the staining process. All samples within the microchannel discs were spun at 2× CLV (∼1200 RPM) for rapid automated image capture over a specified range.

### Flow cytometry analysis

To evaluate the capturing efficiency and purity, depletion assays were performed by counting CD4+ T cells from the aliquots collected before and after the passage of blood through the U-channels and analyzed using a flow cytometer. The samples collected were stained with Multicolor Human T cell marker panel (CD3-FITC, CD4-PE, CD8-APC and CD45-PE/Cy7; Abcam, UK). Prior to flow cytometry analysis, the red blood cells were lysed by incubating with 500 μl MQ water for 30 s and lysis was terminated with the same volume of 2× PBS. The samples were centrifuged at 1500 rpm for 5 min at 21 °C, the supernatant was discarded and the pellets were re-suspended in 500 μl of 1× PBS. Approx. 20 μl of flow count particles (Beckman Coulter, USA) was added to each sample and the flow cytometry data analysis was performed using Kaluza software.

## Result and discussion

### DVD-LSM system capabilities

An integrated DVD-LSM prototype was developed by incorporating a standard DVD reader with rotational control (for sample handling), temperature control (for optimized bioassay), photodiode array (for detection), and software (signal processing and user interface).

The programmable control of the drive's rotation speed is an important design factor for fluidic control in centrifugal microfluidic systems. For example, when using centrifugal force to achieve liquid movement within a disposable disc, a burst valve can be designed to open at a pre-determined centrifugal speed. The standard DVD drive allows control of spin speed at only a few discrete intervals, typically 2× (∼1200 rpm), 4× (∼2400 rpm), 6× (∼3600 rpm), and 8× (∼4800 rpm). These types of valves typically open at rotational speeds of less than 1000 RPM; hence the rotation speeds normally achieved on a DVD drive are not useful. Even though a preferred solution would have been to control the drive's rotation speed by utilizing the built-in motor of the DVD drive, in DVD drives on the market today, the key functionalities are deeply embedded into the hardware or firmware of the drives. Hence, to allow rotational control, we have equipped the prototype with an external motor that attaches to the disc from the top, and provides improved rotation speed control over a large RPM range. The external motor RPM range is dependent on the model of motor. Two different external motors were assembled and tested: a low-speed configuration which provides an improved control in the low-speed range (80–720 RPM) and is useful when control of microfluidic structures such as burst valves is needed, and a high-speed configuration (450–4700 RPM) which is useful when high-speed centrifugation is required (*e.g.* cell sedimentation at a specific RPM).

The DVD-LSM system is equipped with a fan heater that provides temperature control up to approximately 80 °C for use in assays that require elevated temperatures. Examples of such assays include isothermal and other temperature dependent bioassays. A PID temperature controller and three embedded thermocouples are used to monitor and control the enclosure temperature. The temperature in the drive can be held constant to better than ±0.1 °C accuracy for timescales of greater than 60 min. Environmental temperature control makes this system well-suited for installation in remote environments where temperature fluctuations would likely have an impact on diagnostic test result reproducibility. A summary of the hardware specifications for the prototype system developed is given in Table 1.

**Table 1** Integrated DVD-LSM system hardware specifications

| | |
|---|---|
| Weight | 3.5 kg |
| Footprint | $H$ = 18 cm, $W$ = 20 cm, $L$ = 28 cm |
| Power requirements | 240 V AC, < 20 W |
| Measurement wavelength | 650–660 nm |
| Centrifugal spin speed | Low speed motor: 80–720 rpm<br>High speed motor: 450–4700 rpm |
| Temperature control range | RT −80 °C |
| Temperature accuracy | < 0.1 °C |
| Maximum imaging resolution | 0.5 μm × 0.74 μm |
| Radial imaging range | 25 mm–54 mm |

A PCB shown in Fig. 1A has been designed and manufactured to provide integration and control of the additional functionalities of the integrated system. A key function of the PCB is to provide analog to digital conversion of the captured data. The prototype is equipped with a header board with four photodiodes (PDs) placed next to each other to cover as much as possible of the radial distance between the inner and outer edges of the disc, for diameters of 25–54 mm. In some cases, there is a minimal loss of data at the boundary of two PDs, and when switching from one PD to another. This loss of data is due to a small gap region between the PDs that could not be closed, however, longer PDs in future versions of the system will resolve this issue.

### Particle imaging using the developed DVD-LSM system

The use of a DVD drive for imaging is similar to using a standard light microscope; both can generate similar images. The primary difference, for the benefit of the DVD drive, is the ability to integrate various preparative steps on a rotating platform. In order to detect a single cell or particle, the DVD-LSM system scans a designated section of the disc surface, and any object resting or closely positioned on the surface produces a scattered light signal from the DVD laser. The amount of light scattering is detected by a secondary photodetector positioned above the DVD surface. The image resolution of the integrated DVD system was first examined using dried particle slurry samples on the DVD surface. A slurry mixture of 1 μm, 2.8 μm, and 5 μm particles were pipetted onto a non-modified DVD surface and the buffer carrier solution was left to evaporate. The DVD was placed into the DVD-LSM system for automated image capture. As can be seen in Fig. 2, the three sizes of particles are clearly resolved.

The 1 μm particles are clearly resolved in the system and its high resolution capability stems from the drive's optical specifications, and the detection layer of the disc. This resolution is attributed to the linear surface distance between

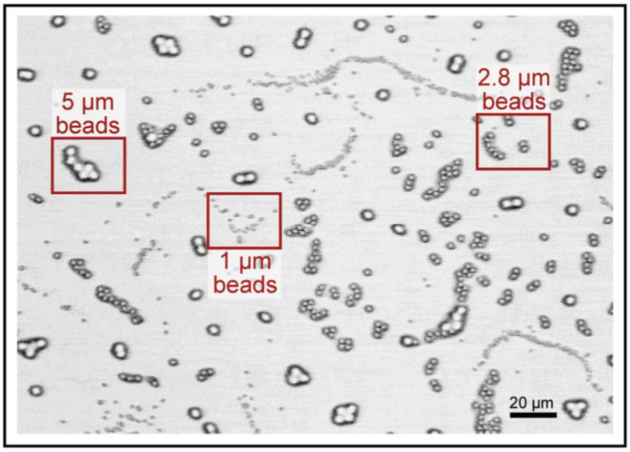

**Fig. 2** High definition DVD-LSM ''image''. Multiple sizes of polymer beads (1 μm, 2.8 μm, and 5 μm) were dried on the DVD surface and imaged using the integrated DVD system. The 1 μm sized beads are clearly resolved in the system. Total scanning time for the surface area was less than 1 s.

each spiral of the track path. For DVD discs the distance is 0.74 μm, a resolution that enables opportunities for various new technologies to utilize this integrated system. For comparison with a commercial CD drive, the resolution would be 1.6 μm based on its optical system specifications (*i.e.* laser wavelength and numerical aperture (NA) of the lens); whereas the blu-ray drive has a shorter laser wavelength and larger NA, resulting in a higher system resolution of 0.32 μm. However, blu-ray drives are not only more expensive components but have a shorter usable focal distance and thus, were not chosen for this prototype system for low-cost diagnostics.

For image based cellular diagnostics, the surface of the half disc has to be modified and bonded to the top fluid structure to form a multi-layer fluidic device. To test UV-bonding of the two half-discs, we initially tested physisorption of biotin molecules on the DVD half disc, followed by UV-bonding of the top fluidic substrate in the PC. The disc surface was modified by pipetting biotinylated molecules onto the DVD surface, capable of capturing streptavidin linked particles. These surface droplets were dried and the half-disc with U-channel features was UV-bonded *via* screen printing of a UV-curable adhesive layer. A particle suspension, containing streptavidin–linked 2.8 μm particles was pipetted into the U-channels in which the access holes were sealed off with transparent tape. The suspension was incubated, and then placed in the DVD-LSM system for automated imaging. The images acquired by the DVD-LSM system are shown in Fig. 3C. The upper portion of the image shows particles in the microchannel specifically bound due to streptavidin–biotin interaction, while the lower portion of the image shows the non-specifically captured particles on the disc surface. In the top portion of the image, streptavidin-biotin bound particles can be clearly resolved without detachment upon spinning the disc at 1200 RPM. The specific attachment of biotin–streptavidin indicates that the industrially scalable UV-bonding technique is compatible with pre-surface modification of the DVD substrate.

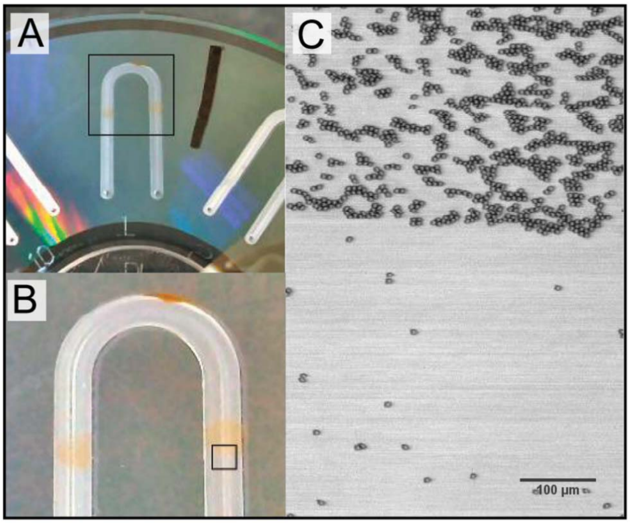

**Fig. 3** High definition DVD image. (A) Injection molded top PC layer with the microchannel features was UV-bonded to the bottom DVD substrate disc with a semi-transparent reflector coating. (B) The simple U-channel design (150 μm × 2 mm) used in this study. (C) DVD Image of 2.8 μm beads specifically attached (streptavidin-biotin interaction) inside a surface-modified microchannel. A drop of solution containing biotin molecules was dried on the DVD surface prior to UV-bonding and streptavidin coated beads were specifically captured and imaged. For clarity, (A and B) are bright field images and (C) is a DVD image. The boxes indicate the image positions.

### Cell capture on a modified surface and imaging

A robust surface modification protocol is imperative for any bioassay, particularly for diagnostic applications. For CD/DVD based assays, the protocol should not affect either the surface or optical path of the detection system. Several groups have demonstrated methods using chemically functionalized polycarbonate surfaces of conventional CDs or DVDs to enable biomolecules binding,[26,27] where the formation of –COOH groups on the PC surface was achieved by UV. However, limited success has been achieved mainly because of the low surface density of reactive groups and strong non-specific adsorption. To develop a bioassay with robust modification chemistry, the DVD discs are coated with a thin layer of $SiO_2$ for surface fuctionalization based on proven silane chemistry. As described in the material and methods section, the DVD half discs were first surface modified with an epoxy-silane chemistry prior UV-bonding as a batch consisting of 25 discs at a time. The surface chemistry is similar to commercially available microarray slides, and the discs could be stored in dry nitrogen bags for several months prior to use. Hence, the modification chemistry developed here is amenable to the scale-up production for cellular and molecular assays.

We used a T2 cell line as a model system to further explore this system for CD4 count based HIV diagnostics. We immobilized anti-CD4 antibodies, and pushed T2 cells through the U-shaped channel using a syringe pump. We obtained single cell resolution images from the DVD system (Fig. 4). The cells are shown to have good coverage over the whole width of the microchannel, demonstrating the efficient

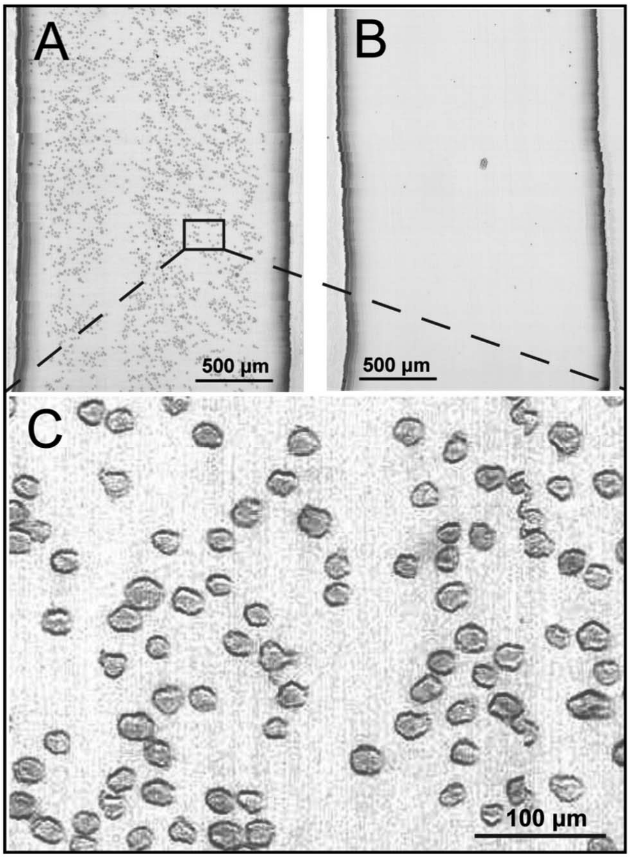

**Fig. 4** DVD platform readout of captured T2 cells (CD4+ and CD8-). (A) Cell capture on the DVD channel coated with anti-CD4 antibodies. The uniform cell coverage over the surface area indicates a good working surface modification protocol. (B) Control using a DVD channel functionalized with anti-CD8 antibodies. The T2 cell lines do not express CD8, and the non-specific binding is very low. (C) Zoomed-in image of the captured cells showing clearly the resolved cells even after digital magnification.

surface modification chemistry and overall surface modification procedure (Fig. 4A). Furthermore, the T2 cells are clearly resolved on viewing at higher magnification, indicating very high quality imaging amenable for counting individual cells (Fig. 4C). The specific T2 cell line used in this study expresses CD4 surface proteins and no CD8 proteins. As a control, we modified the surface of another channel with anti-CD8 antibodies and T2 cells were pumped through the channel. We found very few cells non-specifically captured on the surface (Fig. 4B). These results show that detection with single cell resolution imaging of specifically captured cells are possible in the integrated DVD platform, and should open up the possibilities to develop multiple cellular and molecular based assays. Finally, it is worthwhile mentioning that the DVD-LSM system resolution depends on the resolution settings determined by the user and the quality of light scattering achieved from the object in the focal plane. Hence, the staining procedure increases the amount of scattered light, and results in a clearer image.

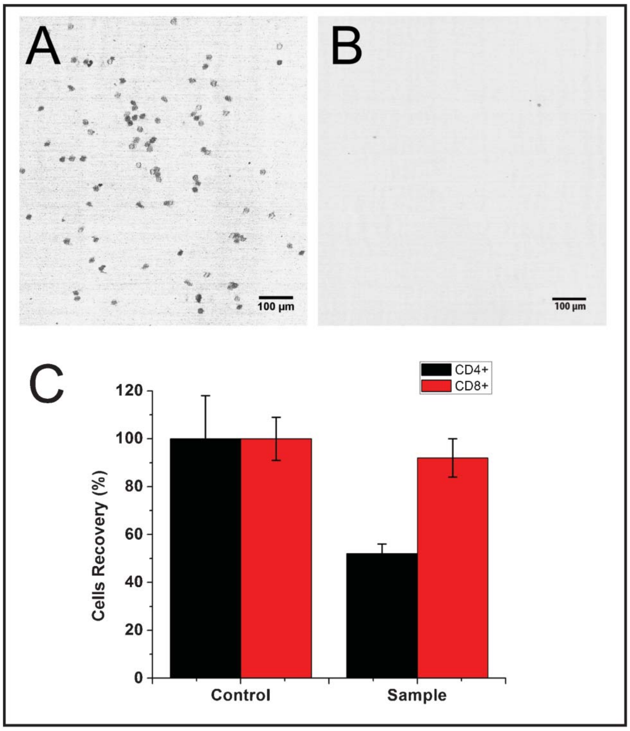

**Fig. 5** DVD platform readout of immuno-captured cells from whole blood. Cell capture on DVD surface coated with (A) anti-CD4 antibody and (B) neutravidin. From the images, CD4+ cells are specifically captured, while there is low non-specific binding of cells. (C) FACS analysis of depletion assay: whole blood analyzed before and after passing through the channel. 50% of CD4+ cells were captured, while 8% of the CD8+ cells were non-specifically captured (*n* = 3).

## CD4+ cell capture from whole blood

Whole blood samples were tested within the DVD-LSM unit as the final step to evaluate this system for potential use in POC HIV diagnostics. BSA solution was pumped through the channels in order to minimize non-specific binding to the surfaces. Whole blood was processed through these channels using a syringe pump. CD4+ cells were captured, stained and imaged (Fig. 5). CD4+ cells were captured onto the anti-CD4 functionalized surface (Fig. 5A), while there were no or few cells captured when the blood is flown through a non-modified channel (Fig. 5B).

As an indirect measure of specificity and yield, we performed flow cytometry analysis of the blood components before and after flowing through the channels. Fig. 5C shows the flow cytometry results, indicating 50% CD4+ cells were depleted from the whole blood. We also measured CD8+ cells for indirect test of specificity and found 8% if these cells were depleted. The overall low capture efficiencies (50%) of the CD4+ sub-types is most likely attributed to the non-optimized channel geometry of the capture channels and flow conditions. More experiments and optimization are required before the system is ready for clinical application.

Previously, Cheng *et al.* investigated CD4+ T cell capture by controlling shear stresses at the chamber walls and found an optimal channel height of 50 μm.[7,8] In this study, we used a channel height of 150 μm, and presumably the cells do not have sufficient time to contact with the capture area. Furthermore, only one side of the channels was deposited with antibodies therefore cells interacting with the top channels were not captured. As cell adhesion is dependent on shear stress, future development work will focus on a full characterization of the effect of radius location on cell capture as well as the rotational speed to wash out non-specifically bound cells. Furthermore, the capture efficiency can be improved by modifying the design of the channels to enhance interaction of cells with the surface. As the HIV assay relies on specific capture and counting, the specificity can be increased by secondary labelling strategy using microspheres coated with anti-CD4 or other visualization strategies such as horseradish peroxidase (HRP) reaction or gold-silver enhancement chemistry. An additional benefit with secondary labelling strategy would enable multiplexing to obtain CD8/CD4 ratio within the same microchannel.

While many of the features presented in Table 1, such as temperature control, rotational control for automated processing of the blood samples, has not been used in the current study, the proposed system has the potential to develop bioassays in a truly integrated Lab-on-DVD system. Alternative CD4 count methods are often based on PDMS substrates that are typically not scalable; optical detection methods require the use of lenses and focusing to analyze the captured cells which increases the cost and portability. The integrated DVD platform presented in this paper benefits from more than 30 years of development in the optical disc industry and delivers an integrated, low-cost, robust and rapid optical detection system with proven sub-micron image resolution.

## Conclusions

We have demonstrated a novel microfluidic system with integrated detection of CD4+ cell counts for HIV diagnostics. A stand-alone low-footprint, point-of-care ''Lab-on-DVD'' system was developed that integrates fluidic propulsion, temperature control, microscopy-based detection and image processing. This system which demonstrated 1 μm image resolution will enable the development of many bio-applications. As a proof of principle for HIV diagnostics, we show specific capture of CD4+ cells direct from whole blood and single cell resolution images. An integrated system is currently under development which will automate the surface modification, sample inlet and washing steps, along with the cell staining steps. This future integrated system, will allow the user to input the blood sample into a pre-modified disc and view the captured cell count results without any additional steps, greatly simplifying the immunocapture detection process. An important feature of the channel design will be to calculate rotational-speed-induced shear stress at various radii along the disc to allow automated washing of non-specific and low binding cells. In addition, we aim to extend this system to handle larger sample volumes, so that low-concentration species (*e.g.* circulating tumor cells) can be handled and processed in a fully integrated sample-to-answer system.

## Acknowledgements

This work has been sponsored in part by the European Commission through the projects FP7 Digital sequencing.

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